PSEUDO-FERTILITY IN NICOTIANA[1]

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Introduction

It has long been known that the eggs of certain hermaphroditic plants and animals cannot usually be fertilized by pollen or sperms from the same individual, and this phenomenon has been termed self-sterility. Although it has been demonstrated in only one animal, *Ciona intestinalis*, the phenomenon has been shown to be fairly widespread throughout the plant kingdom. In 1895, Knuth listed 134 observed self-sterile species of Angiosperms belonging to 46 families, and East and Park ('17) estimated that 70 per cent of these observations proved definitely that the species were self-sterile. Hence, as early as 1895, at least 100 self-sterile species of Angiosperms had been observed.

In *Nicotiana*, the primary difference between sterile and fertile combinations has been shown (East and Park, '18) to be the difference in rate of pollen-tube growth. In incompatible combinations, the pollen grains germinate but do not grow with sufficient rapidity to reach the ovary before the flower falls. They showed, through cytological studies of styles taken at successive twelve-hour intervals after pollination, that the pollen-tubes of fertile combinations exhibit an acceleration in their rate of growth, their growth curves when plotted being similar to those representing autocatalytic reactions. On the other hand, the pollen-tubes of sterile combinations maintain a constant rate of growth, showing no acceleration, their growth curves being straight lines. By virtue of the acceleration in the rate of growth, the pollen-tubes of compatible matings reach the ovary in less than 96 hours after pollination, whereas in incompatible matings they fail to reach the ovary within the life of the flower.

[1] An investigation carried out at the Missouri Botanical Garden in the Graduate Laboratory of the Henry Shaw School of Botany of Washington University and submitted as a thesis in partial fulfillment of the requirements for the degree of master of science in the Henry Shaw School of Botany of Washington University.

Cross-incompatibility among self-sterile individuals was not described in the early work on self-sterility, and it was not until the work of de Vries ('06) on the self-sterile species *Linaria vulgaris* that the existence of intra-sterile, inter-fertile classes was first observed. That is, de Vries demonstrated the fact that there were among the self-sterile individuals of *Linaria vulgaris* with which he was working, two classes, and that every individual belonging to one class was cross-sterile with every individual of the same class and cross-fertile with every individual of the other class. Since that time, such intra-sterile, inter-fertile classes have been observed in various species by Correns ('12, '16), East and Park ('17), Baur ('19), Lehmann ('19), Crane ('23), Shull ('23), and Anderson ('24). East and Park ('17) showed that this cross-sterility is identical in nature with self-sterility, and that an interpretation which holds for one phenomenon must likewise hold for the other.

However, among self-sterile plants, individuals exhibiting self-fertility to some degree are frequently found. East and Park ('17) occasionally obtained, in their self-sterile hybrids between *Nicotiana alata* and *N. Forgetiana* and in several self-sterile species of *Nicotiana*, small capsules which contained relatively few seeds and rarely, if ever, approximated the size of those resulting from fertile combinations. It has been agreed by all investigators of the subject that a plant is self-fertile if it sets a full capsule of seeds when pollinated with its own pollen, and self-sterile if no seeds are set after self-pollinations. Neither of these two categories, however, includes those individuals of self-sterile species which exhibit a small but varying amount of self-fertility, and such plants have been described by East and Park as being "pseudofertile."

This phenomenon has been described, although in some cases not by this name, by Correns ('12, '13, '16) in the self-sterile species *Cardamine pratensis* and *Linaria vulgaris;* East and Park ('17, '18) in several species of *Nicotiana* and in hybrids between *Nicotiana alata* and *N. Forgetiana;* Sutton ('18) in plums and cherries; Baur ('19) in *Antirrhinum hispanicum;* Lehmann ('19) in *Veronica syriaca;* Shull ('23) in *Bursa grandiflora;* Anderson ('24) in *Nicotiana alata*, *N. Forgetiana*, and hybrids between *N.*

alata and *N. Forgetiana;* and Smith ('24) in hybrids between *N. alata* and *N. Forgetiana.*

East and Park ('17) observed the occurrence of pseudo-fertile individuals particularly among plants which were nearing the end of the flowering season, and hence exhibiting weakened vigor. By comparing the curve of pollen-tube growth in a pseudo-fertile combination with that in a self-sterile mating, East and Park ('18) found indication of the possibility that the so-called phenomenon of pseudo-fertility was a variant of self-sterility which was brought about by unfavorable environmental conditions and general decrease in the vigor of the plant. That is, there was apparently little or no acceleration in the rate of growth of the pollen-tubes such as is found in normal fertile combinations, but the growth was merely more rapid throughout the course of the pollen-tube through the style.

Stout ('16, '17), in connection with his studies on self- and cross-pollinations in *Cichorium Intybus*, observed a considerable variation in the expression of self-sterility and attributed it to germinal disturbances, which, however, he concluded were too variable to permit of any type of Mendelian interpretation. This feeble or "partial compatibility," as he terms it, he believes "manifests itself quite indiscriminately throughout the entire period of bloom" and is independent of any decrease in vegetative vigor. Hence, according to him, end-season fertility is comparatively rare and is not a condition commonly operating in incompatible plants. It is quite possible that he was observing in *Cichorium Intybus* the same phenomenon which was termed "pseudo-fertility" by East and Park ('17) in their work on *Nicotiana* hybrids, and that he has failed to distinguish between pseudo-fertility and true self-fertility. That is, it is possible that the "sporadic development of self-compatibility giving self-fertility among the progeny of self-sterile lines of descent" which he observed may be due to other changes rather than germinal disturbances. However, it is true that if the variations which he finds among self-sterile individuals are expressions of pseudo-fertility rather than of true fertility, the degree of pseudo-fertility must be much higher and much more variable in *Cichorium Intybus* than it is in *Nicotiana alata, N. Forgetiana,* and hybrids between them.

Stout ('20) reported results of continued work on *Cichorium Intybus* and of preliminary work on other genera. In *Cichorium Intybus,* he failed to find any progressive seasonal increase in pseudo-fertility. In *Brassica pekinensis* he observed a few cases of mid-seasonal fertility. *Eschscholtzia californica* was found to be somewhat pseudo-fertile, one plant in 200 setting a capsule comparable to those resulting from fertile pollinations, whereas *Raphanus sativus* was still more self-sterile, only one plant in 200 showing any indication of pseudo-fertility.

Sutton ('18) concludes that in plums and cherries the results are "consistent with the supposition that the plants consist of two larger classes, self-fertiles and self-steriles, with a smaller number of plants of intermediate properties." For this smaller group, she offers two possible interpretations. The intermediate group and some of the self-fertiles may be supposed to be heterozygous, and the self-steriles, homozygous, the occasional indications of partial self-fertility among the latter being attributable to errors probably. On the other hand, when a few fruits are formed out of a large number of pollinated flowers, the fact may mean that compatibility exists in a very slight degree. "If this could be confidently asserted," she adds, "it would be tempting to suppose that the tree may be a mosaic in that respect."

It is this phenomenon of pseudo-fertility, defined by East and Park ('17) and described by numerous investigators before and since, with which the present paper is concerned. In the course of this investigation on *Nicotiana alata* and on hybrids between *N. alata* and *N. Forgetiana,* a comparative measure of pseudo-fertility has been developed, by means of which it has been possible to demonstrate that pseudo-fertility in self-sterile matings is of the same order as that in cross-sterile matings, that pseudo-fertility is of a different order from true fertility but of the same order as self-sterility, and that in genetic strains concerned, the phenomenon was but slightly affected by environmental changes, or with progress of the flowering season. Measurements of the pollen grains were made and indications were found that the percentage of variability in diameter is significantly greater in plants resulting from self-pollinations than in those coming from cross-pollinations.

Materials and Methods

In the present series of investigations on the phenomenon of pseudo-fertility, *Nicotiana alata* Lk. and Otto var. *grandiflora* Comes, and F_7 and F_8 hybrids between *N. alata* and *N. Forgetiana* were used, the seed coming originally from stock used in investigations on self-sterility at the Bussey Institution of Harvard University (see East, '15; East and Park, '17; and Anderson, '24). Strains of the pure species and of the hybrids coming from seeds planted in September, 1922, were taken over by the author in January, 1923, and during the next 18 months the following investigations were conducted on that and the succeeding generation of plants.

The material was particularly satisfactory for such a series of investigations. Capsules containing, on an average, between 300 and 500 seeds each were set in 95 per cent of the compatible combinations; and thus pseudo-fertility, in which usually only a few seeds are set at more or less infrequent intervals, was readily distinguished from true fertility. The plants grow well under greenhouse conditions, and they can be cut back and made to pass through a second flowering season, if so desired. However, due to the attacks of mosaic and to the extremely hot summers, it is almost impossible to carry the plants over from one season to the following in this climate.

Experimental error due to the contamination of pollen or to accidental pollination of flowers was reduced as far as possible. With the anthesis of the first few flowers of a plant, the panicle was enclosed in a paper bag so as to prevent contamination or cross-pollination by wind or insects. When the plants were unbagged for the purpose of pollinating the flowers, care was taken that pollen from one plant was not permitted to fall on the flowers of near-by plants. In making pollinations, newly opened flowers were used in order that there might be as little danger as possible of contamination by foreign pollen. The pollinations were effected by carefully dusting the stigma of a given flower with the desired pollen. When necessary, the flowers were emasculated before being pollinated. Also, after each emasculation or pollination the hands and forceps were washed in 95 per cent alcohol in order to kill all the pollen. That this procedure

was effective in the prevention of contamination by foreign pollen was demonstrated by Anderson ('24), as follows: The fingers were dusted with pollen and 4 pollinations made. Then, after rinsing the hands with alcohol, 4 more pollinations were made on the same plant with the remaining pollen. Full capsules were set in the first 4 pollinations and none in the last, thus showing that the alcohol was efficient in destroying the pollen grains.

Presentation of Data and Discussion

SELF-STERILITY, SELF-FERTILITY, AND PSEUDO-FERTILITY

As has already been stated, the object of these investigations has been a study of pseudo-fertility, particularly with respect to the relation which it bears to self- and cross-sterility, and cross-fertility. It has been indicated by East and Park ('18), through a study of the relative rates of pollen-tube growth, that pseudo-fertility in *Nicotiana* is of the nature of true sterility rather than of that of true fertility. That is, their data indicated that the phenomenon was probably a variant of true sterility, brought about by unfavorable environmental conditions, and not a modified expression of true fertility resulting from germinal modifications in a self-sterile race. The present series of investigations was designed to prove definitely, by a method other than that of pollen-tube growth, that, in *Nicotiana*, pseudo-fertility is of the order of self-sterility and not of self-fertility.

The method used centers about the fact which had previously been observed by Anderson that pollinated unopened buds will frequently exhibit pseudo-fertility when mature flowers will not. With this idea in mind, series of pollinations were made simultaneously on unopened buds, and first and second flowers of the same branch of the panicle (fig. 1). The flowers were numbered from apex to base of the branch of the panicle, thus making the first the youngest of the mature flowers. The series of pollinations were made and the panicles bagged. After one week, those flowers, the ovaries of which showed no enlargement or indication of setting seed, were removed. This was justified by the previously determined fact that certain indication of capsule formation appears within the first week after pollination. Those flowers which indicated that seeds were being set were allowed to

remain on the plant until the capsule showed signs of dehiscence, at which time they were removed. When thoroughly dry, the seeds in each capsule were counted and recorded. As there was not sufficient time to allow those capsules formed during the latter part of the work to mature on the plant, they were removed two

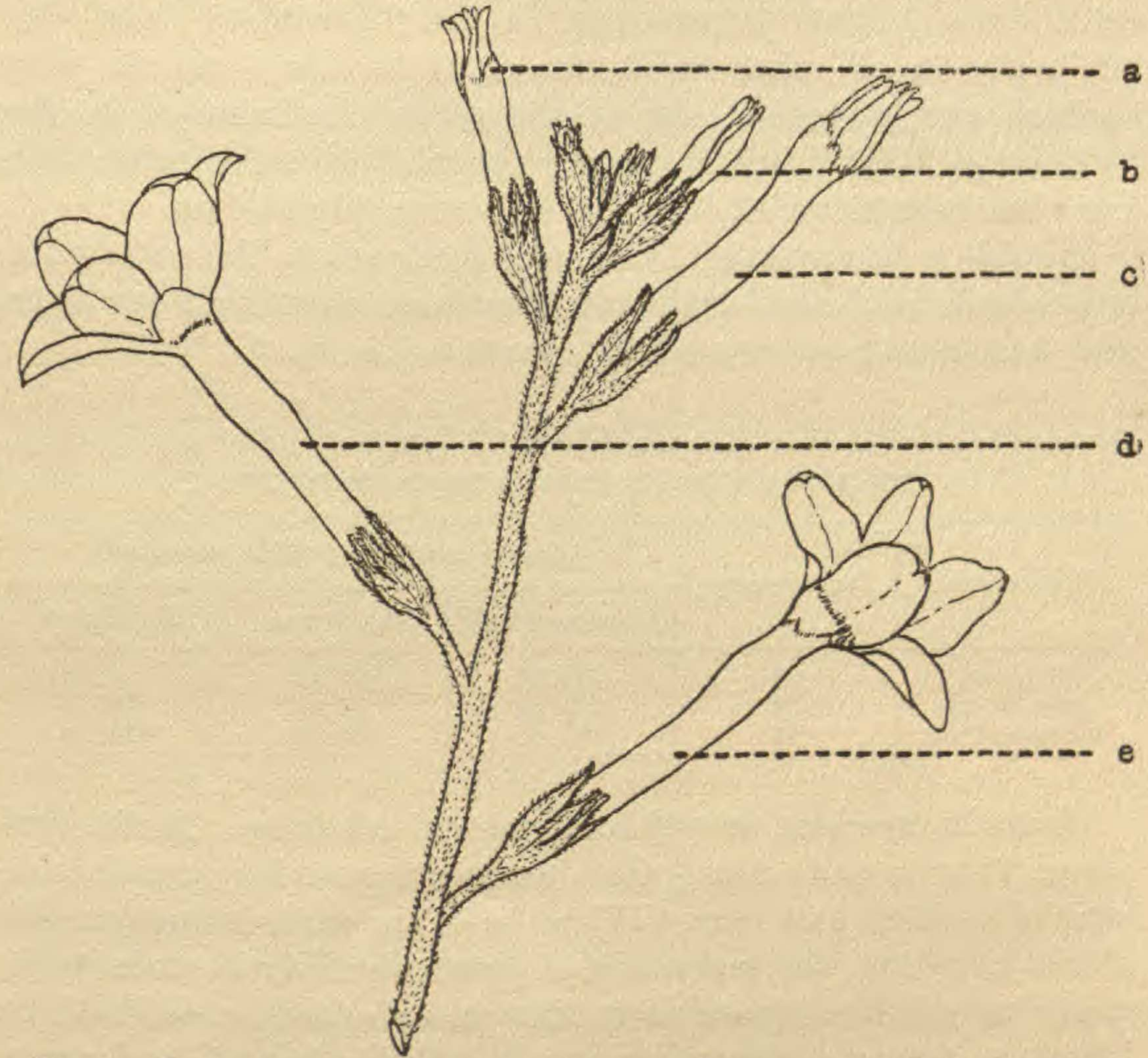


Fig. 1. Typical branch of a panicle: a, young bud; b, very young bud; c, unopened bud; d, first flower; e, second flower.

weeks after the pollinations were made and were dried in an oven at 100° C. for 30 minutes. This heating served to dry the seeds so that they could be readily separated and counted, and was possible by virtue of the fact that the chief aim was a determination of the number of seeds set, and that the seeds were not needed for the plants of the next season.

Such series of pollinations were made on both the hybrids and the pure species, *Nicotiana alata*, but at this point only those

results obtained from the hybrids are of interest. Two hundred thirty-three series, including self-sterile, cross-sterile, and cross-fertile combinations were made on the hybrid plants. For the resulting data see "Complete Data" at the close of this paper. In several instances, capsules were formed and were lost before the seeds were counted (represented in the "Complete Data" by "?"), but in all other cases, the seeds of every capsule were counted and recorded. All of the series are included in the "Complete Data," but any series containing one or more such cases has been excluded from the following calculations.

In table I, the average number of seeds set in the self-sterile, cross-sterile, and cross-fertile combinations respectively are given and these results are graphically presented in fig. 2.

TABLE I

PSEUDO-FERTILITY VERSUS TRUE FERTILITY

Pollinations	No. of series	Average number of seeds per capsule		
		Unopened bud	1st flower	2nd flower
Self-sterile	84	86.36	27.92	5.63
Cross-sterile	63	138.65	32.16	18.88
Cross-fertile	41	439.46	465.83	414.80

Figure 2 expresses several fundamental relations. In the first place, it shows quite clearly that just as cross-sterility was demonstrated by East and Park ('17) to be of the same nature as self-sterility, so here, the expression of pseudo-fertility in cross-sterile combinations is comparable to that in self-sterile combinations. The two curves representing pseudo-fertility in self- and cross-sterile combinations are essentially the same and are in relatively the same position on the graph. The only interpretation which can be applied to this close similarity between these two curves is that pseudo-fertility in cross-sterile combinations is of the same order as that in self-sterile pollinations.

The second and probably the most fundamental relation to be obtained from table I and the curves in fig. 2 is that pseudo-fertility is not of the nature of true fertility. The curve representing true fertility is not only of a different type from those representing pseudo-fertility, but it occupies an area on the

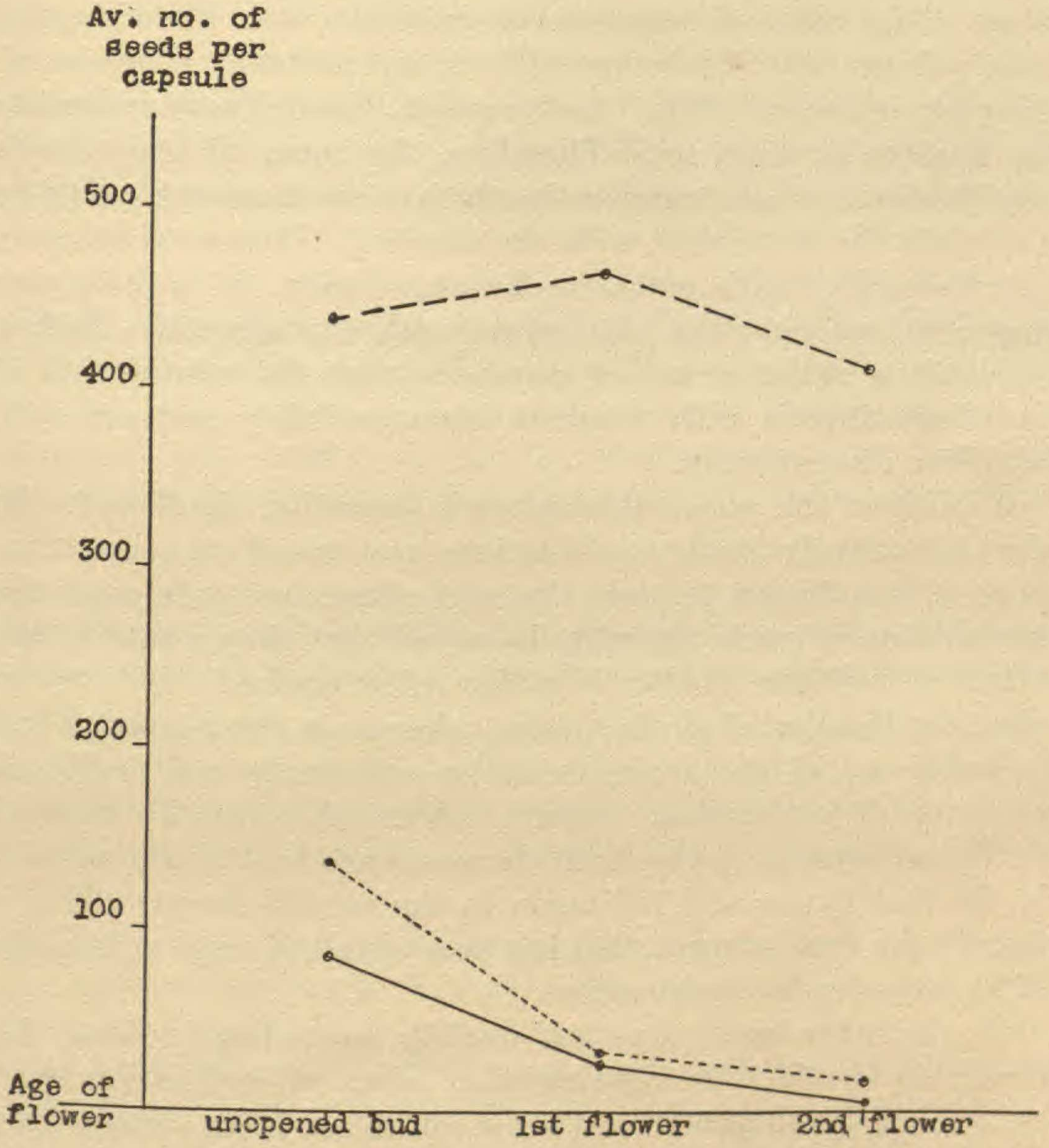


Fig. 2. Pseudo-fertility versus true fertility: ———, self-sterile pollinations; ·············, cross-sterile pollinations; — — — —, cross-fertile pollinations.

graph which is widely separated from that occupied by the curves representing pseudo-fertility. In effect, the lowest point on the curve of the former is practically three times as high as the highest point of either of the latter two curves. It is indeed quite obvious that pseudo-fertility as expressed in the hybrids between *Nicotiana alata* and *N. Forgetiana* cannot be of the same order as true fertility.

Just as it is obvious even from a casual examination of fig. 2 that pseudo-fertility in either self- or cross-sterile combinations

is not of the order of complete compatibility, it is likewise quite clearly shown that it is comparable to, and probably a variant of, complete incompatibility. In the graph, the ordinate represents the number of seeds set. Therefore, the curve of truly sterile combinations, or combinations in which no seeds are set, would be a straight line coincident with the abscissa. Hence, to compare pseudo-fertility with complete incompatibility, it is necessary merely to compare the two curves with the abscissa. Such a comparison indicates rather decidedly that the relationship of pseudo-fertility is with complete incompatibility and not with complete compatibility.

A study of the curves themselves is somewhat significant. In the case of truly fertile combinations, one would expect pollinations of first flowers to yield the most abundant seeds, since the second flowers would probably be so old that they would wither before a sufficient number of pollen-tubes reached the ovary to effect fertilization of all the ovules, whereas in the unopened bud probably not all the ovules would be sufficiently well developed to permit of fertilization. Hence, one would expect the curve of fertile pollinations to rise from the unopened bud to a maximum in the first flower and fall again in the second flower. This is exactly the type of curve that has been obtained from an average of 41 cross-fertile combinations.

On the other hand, since self-sterility (according to East, '15) is due to the fact that pollen-tubes after self-pollination show no acceleration in growth and hence fail to reach the ovary within the life of the flower, it would be expected that pollinations of unopened buds would yield the most seeds by virtue of the fact that in such pollinations additional time is gained and the pollen-tubes may reach the ovary before the flower falls. That this is true, is quite evident in the curve which shows a sudden drop from the unopened bud to the first flower followed by a gradual decline to the second flower. This is further evidence that the pseudo-fertility of self- and cross-sterile combinations is not of the same order as true fertility.

In this connection, observations made on a single plant, WA-1, are of interest. This plant was a *Nicotiana alata* × *N. Forgetiana* hybrid of a genetic strain other than that to which the remainder

of the plants used belonged and hence not included in the "Complete Data." It was apparently completely self-sterile, no seeds being set in 17 series of pollinations made on unopened buds, first and second flowers. Series were pollinated at intervals throughout the flowering season of the plant, which, in this case, extended over a period of 50 days, and there was no evidence whatsoever of an end-seasonal pseudo-fertility. It was, however, possible to obtain seeds in considerable numbers by pollinating buds from 4 to 6 days before anthesis, as shown in table II. Hartley ('02), in attempting premature fertile pollinations in *Nicotiana Tabacum*, was not only unable to get the flowers to set seed, but also found that the growth of the pollen-tubes into the ovaries before the ovules were sufficiently mature for fertilization resulted in an injury which caused the flowers to fall immediately. In view of his observations, it is interesting that in these sterile combinations, seeds can be set in considerable numbers by pollinating the very young buds.

In the light of this comparison between the curves representing true fertility and the degree of pseudo-fertility as indicated by the number of seeds set in the unopened bud, first and second flowers, we might consider the number of seeds set in younger buds and older flowers. In cross-fertile combinations seeds have been set in the third and fourth flowers in the few cases where they have been tried, but always fewer than in the second flower. Therefore, as the flower ages, fewer and fewer seeds are set until theoretically we reach an age at which no seeds are set. At this place, the curves representing true fertility and pseudo-fertility would be coincident with each other and with the abscissa. Likewise, we can conceive of a point at the opposite end of the curves where they would pass through the same point. As younger and younger buds are pollinated in sterile combinations, more and more seeds are set and the curve for pseudo-fertility rises. As will be shown in the case of WA-1, plants which are apparently completely self-sterile when pollinated in the unopened bud, first and second flowers may be pseudo-fertile to a considerable degree if pollinations on younger buds are made. However, in cross-fertile matings, fewer seeds were set in unopened buds than in the first flowers. As younger buds would be pollinated, it seems probable

that fewer and fewer seeds would be set. In effect, Hartley ('02) found in *Nicotiana Tabacum* that very early fertile pollinations caused the flowers to fall before any seeds were set. Hence, we can conceive of the curve for true fertility falling off rather abruptly, and in the drop it would probably cross the rising curve for pseudo-fertility. Therefore, if the two curves would be carried out sufficiently far in either direction it is very probable that they would meet. This point should be considered in any attempt to distinguish between pseudo-fertility and true fertility, and the phenomena should be studied on flowers at that age at which the difference is greatest.

TABLE II

PSEUDO-FERTILITY IN PREMATURE POLLINATIONS

Pollination	Date	No. of seeds per capsule		
		Very young bud	Young bud	Unopened bud
WA-1 selfed	10-24-23	0	0	0
WA-1 selfed	10-24-23	150+	0	0
WA-1 selfed	10-24-23	37	0	0
WA-1 selfed	10-24-23	0	0	0
WA-1 selfed	10-24-23	432	0	0
WA-1 selfed	11- 3-23	319	0	0
WA-1 selfed	11- 3-23	352	0	0
WA-1 selfed	11- 3-23	487	0	0
WA-1 selfed	11-12-23	0	6	0
WA-1 selfed	11-19-23	0	454	0
WA-1 selfed	11-19-23	440	0	0
WA-1 selfed	11-24-23	0	391	0
WA-1 selfed	11-24-23	167	0	0
WA-1 selfed	12- 7-23	0	0	0
WA-1 selfed	12- 7-23	0	150	0
WA-1 selfed	12-13-23	0	0	0

In the table, buds before anthesis are termed young buds and those 6 days before anthesis are termed very young buds. It is of interest to find that seeds can be set by pollinations made so early in the bud, and the fact may prove to be of practical significance in obtaining seeds from normally incompatible combinations. However, before its practical significance could be asserted, it would be necessary not only to run germination tests on the seeds produced by such early pollinations to see whether or not they are fertile, but to discover the extent to which it is possible to obtain seeds by this means from other Angiosperms.

As has been said earlier, the panicles were placed in paper bags so as to avoid cross-pollination by wind or insects. In view of the fact that environmental factors have been thought to increase pseudo-fertility, the question arose as to whether the bagging of the panicles in any way influenced the degree of pseudo-fertility expressed by the plant. In order to determine any such possible influence, the following experiment was carried out.

Two series were made simultaneously on different branches of the same panicle of the plant. The branch bearing one series was bagged, whereas that bearing the other was not included. To avoid accidental cross-pollination in the case of those not bagged, the corollas of the flowers were tied, and in that way no foreign pollen could reach the stigma. Ten such series, including both self- and cross-sterile combinations, were made on different plants, the data of which are given in table III.

TABLE III

INFLUENCE OF BAGGING ON PSEUDO-FERTILITY

Pollination	Date	Number of seeds per capsule					
		Unopened bud		1st flower		2nd flower	
		Bagged	Unbagged	Bagged	Unbagged	Bagged	Unbagged
CA-4×CB-13	4-19-23	338	158	0	0	85	0
CC-1×CC-5	4-26-23	355	461	390	381	338	261
CC-5×CC-1	4-26-23	0*	0	0	?	0	?
CB-4×CG-8	4-19-23	230	292	87	105	41	0
CG-20×CL-4	4-19-23	339	0	0	0	0	0
CC-1 selfed	4-26-23	0	0	0	0	0	0
CC-5 selfed	4-26-23	0	0	0	0	0	0
WA-1 selfed	10-19-23	0	0	0	0	0	0
WA-1×WB-1	10-19-23	0	0	0	0	0	0
WB-1 selfed	10-20-23	0	0	0	0	0	0
Average		140.2	101.22	53	54	52.6	29

* This series not included in the averages because of the lost capsules in the unbagged first and second flowers.

A comparison of the average number of seeds set in the bagged series with that in the unbagged, together with a comparison of their respective graphs (fig. 3), shows quite clearly that bagging does increase the degree of pseudo-fertility. Considering together the average number of seeds set in unopened bud, first and second flowers, there has been an increase of 33.42 per cent in the

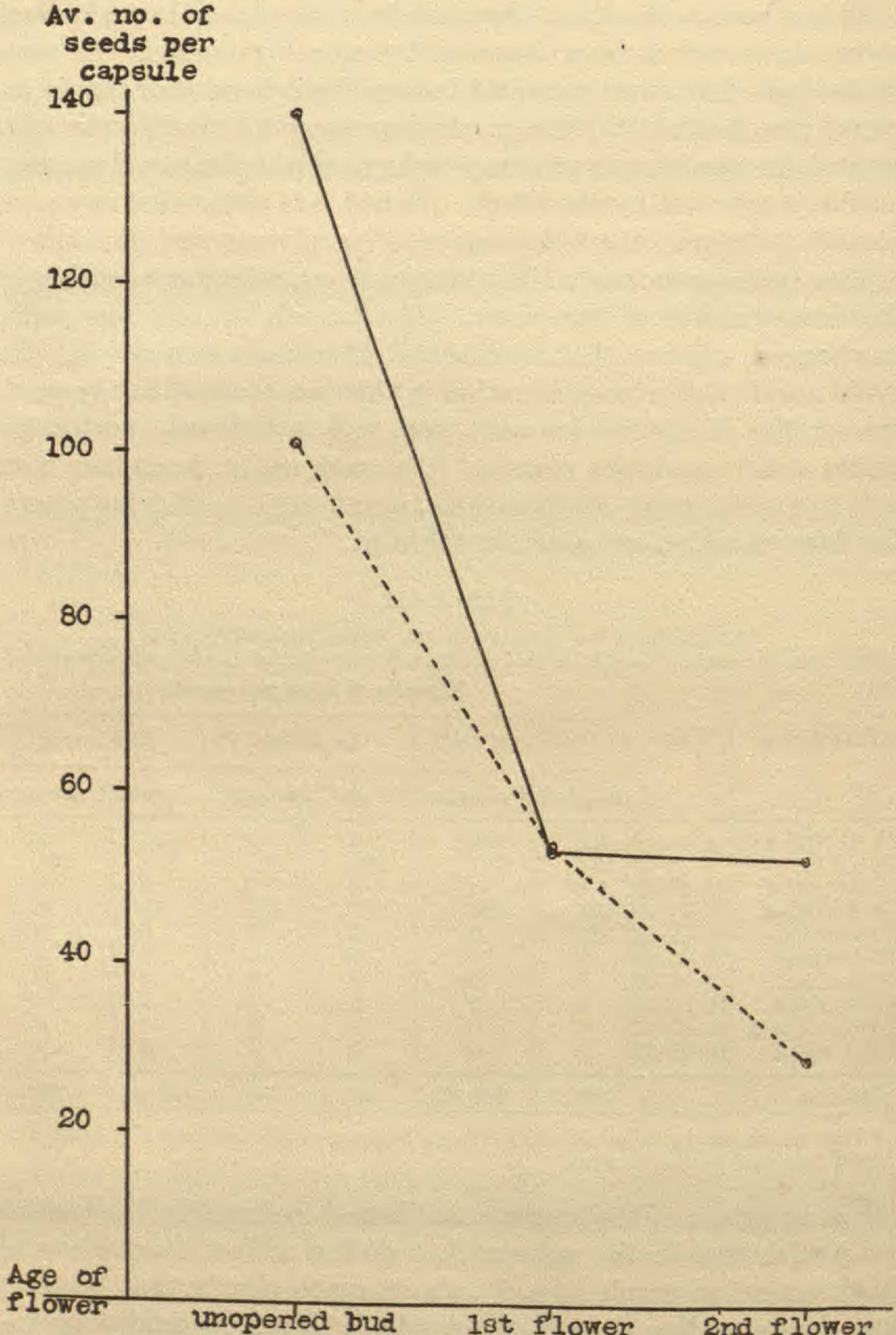


Fig. 3. Influence of bagging on pseudo-fertility: ———, bagged flowers; ...., unbagged flowers.

number of seeds set in the bagged over those in the unbagged panicles. The fact that pseudo-fertility is thus increased by bagging must be remembered in considering the actual values of the data as given. However, bagging was practised throughout the entire series of pollinations and hence the relative values still remain true. The error resulting from bagging the flowers is constant throughout the experiments and hence does not affect the conclusions which are to be drawn from the data recorded.

INFLUENCE OF SEASONAL PROGRESS

East and Park ('17) suggested that pseudo-fertility was a variant of self-sterility brought about by unfavorable environmental conditions which caused a decrease in the vigor of the plant. Particularly did they find pseudo-fertility near the end of the flowering season. With this idea in mind, successive series of self-pollinations were made on 16 individuals during the early, and then during the latter part of their flowering season. The flowering season of the plants grown in the Bussey Institution was sometimes as long as 3 months, but in our greenhouses this was not the case. Only rarely did the flowering season of any one plant continue for more than 3 weeks, and usually it lasted only 10 days or 2 weeks. Hence, with the exception of the plant WA-1 previously cited, the interim between the early and later pollinations was a week to 18 days. The data from these repeated series are given in table IV.

TABLE IV

SEASONAL CHANGE IN PSEUDO-FERTILITY

Pollination	Date	Number of seeds per capsule		
		Unopened bud	1st flower	2nd flower
GOB-4 selfed	4-21-24	0	0	0
GOB-4 selfed	4-30-24	314	0	0
GA-2 selfed	4-18-24	0	0	0
GA-2 selfed	4-30-24	27	0	0
GC-3 selfed	4-14-24	124	0	0
GC-3 selfed	4-26-24	387	0	0
GC-6 selfed	4-18-24	0	0	0
GC-6 selfed	4-26-24	149	0	0

TABLE IV (*Continued*)

Pollination	Date	Number of seeds per capsule		
		Unopened bud	1st flower	2nd flower
GF-1 selfed	4-16-24	0	0	0
GF-1 selfed	4-28-24	329	0	0
GF-1 selfed	4-30-24	0	0	0
LH-1 selfed	4-21-24	67	0	0
LH-1 selfed	4-28-24	45	79	0
CB-14 selfed	3-27-23	473	136	48
CB-14 selfed	4-14-23	550	29	54
GC-1 selfed	3-29-24	48	0	0
GC-1 selfed	4- 8-24	14	0	0
GF-2 selfed	4-16-24	121	0	0
GF-2 selfed	4-28-24	0	0	0
GF-9 selfed	4-21-24	0	208	0
GF-9 selfed	4-28-24	0	0	0
GOA-2 selfed	4-16-24	419	154	0
GOA-2 selfed	4-30-24	7	0	0
GOF-3 selfed	4-18-24	0	0	0
GOF-3 selfed	4-30-24	0	0	0
GE-1 selfed	4-21-24	0	0	0
GE-1 selfed	4-28-24	0	0	0
GOA-1 selfed	4-11-24	0	0	0
GOA-1 selfed	4-30-24	0	0	0
GOA-3 selfed	4-18-24	0	0	0
GOA-3 selfed	4-30-24	0	0	0
GOA-4 selfed	4-21-24	0	0	0
GOA-4 selfed	4-30-24	0	0	0

The possible change in degree of pseudo-fertility with seasonal progress is best studied by comparing averages (see table v) of the number of seeds set in the early part of the season with those in the later pollinations. From this table and fig. 4 it appears evident

TABLE V

SEASONAL CHANGE IN PSEUDO-FERTILITY

	Average number of seeds per capsule		
	Unopened bud	1st flower	2nd flower
Early pollinations	78.25	31.125	3
Later pollinations	107.625	6.75	3.375

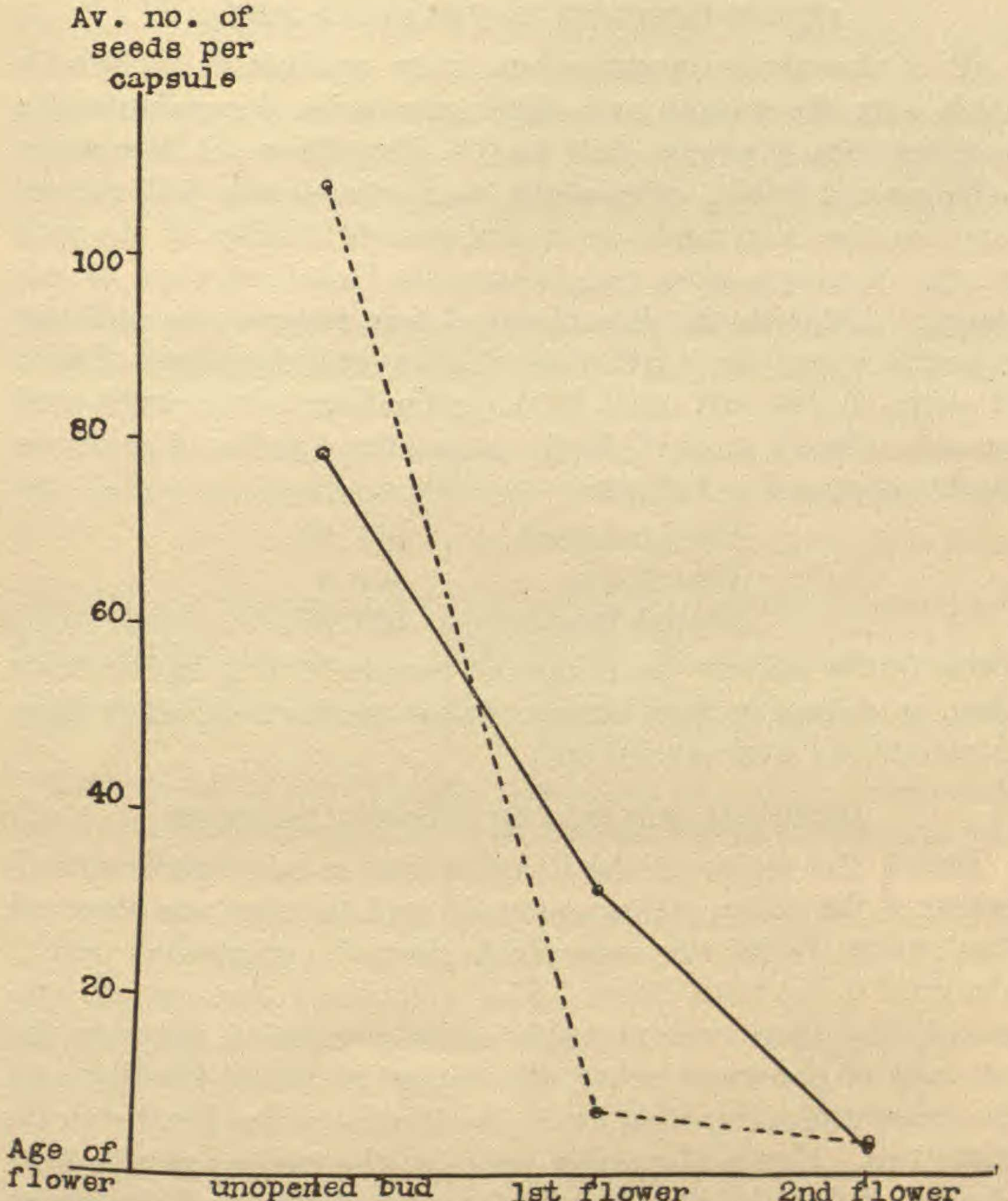


Fig. 4. Seasonal change in pseudo-fertility: ———, early pollinations; ...., later pollinations.

that, considering all 3 pollinations of each series, there was very little change of pseudo-fertility with the progress of the season. The average number of seeds set in the later series is only 4.78 per cent greater than that in the early pollinations and this is scarcely great enough to be significant. Hence, there was little if any seasonal change in pseudo-fertility shown in the *Nicotiana alata* × *N. Forgetiana* hybrids used in the present investigations.

PSEUDO-FERTILITY IN NICOTIANA ALATA

All of the above investigations were conducted on hybrids which were the seventh and eighth generation descendants of a cross between *Nicotiana alata* and *N. Forgetiana*. A few series, including self-sterile, cross-sterile, and cross-fertile pollinations, have likewise been made on several genetic families of the pure species, *Nicotiana alata* (see "Complete Data" at close of this paper). Although the data obtained here may not be sufficient to permit of positive conclusions, it does seem significant that in 22 series of self- and cross-sterile pollinations not a single seed was set, whereas in the 6 fertile crosses the number of seeds per capsule averaged as follows:

Unopened bud	511.83
First flower	409.5
Second flower	521.67

These results indicate an absence of pseudo-fertility in *Nicotiana alata*, or at least in those strains of that species with which these investigations were carried out.

ABNORMAL POLLEN AND SELF-POLLINATIONS

During the course of the investigation, a microscopic examination of the pollen grains was made, and the fact was observed that among the hybrids some plants showed a greater per cent of abnormal pollen than others. The preliminary observations suggested that there was probably some correlation between the per cent of abnormal pollen, the degree of pseudo-fertility, and the amount of self-pollination in the history of the genetic strain concerned. Hence, the pollen grains of the various strains were studied, and in the case of each plant examined the diameters of 50 pollen grains, taken directly across the field of the microscope so as to insure a random sample, were measured by means of an eye-piece micrometer (see "Complete Data" for the resulting measurements).

Pollen of plants containing a considerable amount of small pollen grains likewise contains an approximately equal number of unusually large grains, since, due to the abortion of some of the grains, others are given the opportunity of an unusual degree of development. Hence, in considering the measurements of 50

grains, those plants with a low percentage of abnormal pollen have many grains near the mean diameter, with very few, if any, at the extreme. On the other hand, those plants with a high percentage of abnormal pollen have fewer grains of the mean diameter and more at the extremes than do those with a low percentage. Consequently, the relative per cent of abnormal pollen can be judged by the relative height and spread of the curves, which can in turn be measured by the standard deviation, σ. The standard deviation, then, can be used as a basis for comparison of the amount of abnormal pollen in any two groups of plants, and by this means it is possible to determine whether or not there is a significant difference between the two strains.

Class	Genetic families included
Selfed of selfed	LA, LC, LE
Selfed of crossed	CA, CB, CC, CD
Crossed of selfed	GOB
Crossed of selfed and crossed	GA, GC, GOF, GOX
Crossed of crossed	CL, CM, CG, CH, GE, GF, GOA, GOC, LH

Each group of families was considered as a unit. Frequency tables were made for each of them, and the standard deviation was calculated in every case with the following results.

Class	No. of grains	Standard deviation
Selfed of selfed	450	$.9933 \pm .0223$
Selfed of crossed	475	$1.0834 \pm .0237$
Crossed of selfed	350	$.7032 \pm .0179$
Crossed of selfed and crossed	1500	$.6098 \pm .0075$
Crossed of crossed	1300	$.7296 \pm .0097$

The notation must be made here that the measurements in the selfed of crossed families and in a few of the crossed of crossed families were made early in the work and only 25 grains were measured in each plant. The latter have not been brought into this calculation, but since there were no measurements made on the selfed of crossed families during the latter part of the work, they are included here.

Since it was conceived that possibly the abnormal pollen was correlated with the degree of self-pollination in the history of the plants or with the recency of the self-pollination, the plants were divided into the following groups on this basis. The "selfed of

selfed" class came from seeds which were the result of self-pollinations made on plants which, in turn, were the results of self-pollinations. The "selfed of crossed" class came from self-pollinations on plants which were the product of a cross-pollination. The "crossed of selfed" class were plants which were the product of crosses between plants which, in turn, had come from self-pollinations. The "crossed of selfed and crossed" class were those individuals coming from crosses between two plants, one of which was the result of a self-pollination and the other the product of a cross between two parent plants. Finally, the "crossed of crossed" plants were the product of crosses between plants which, in turn, were the product of cross-pollinations.

To determine the significance of the difference it is simply necessary to divide the difference in the values of σ by the probable error of that difference, and if the quotient is greater than three the difference is significant.

$\text{P.E.}_{\text{diff.}} = \sqrt{E_{\sigma_1}^2 + E_{\sigma_2}^2}$ where E_{σ_1} = probable error of σ in one group and E_{σ_2} is that of σ in the other. Therefore,

$$\frac{\text{Diff.}}{\text{P.E.}} = \frac{\text{Diff.}}{\sqrt{E_{\sigma_1}^2 + E_{\sigma_2}^2}}.$$

By means of this equation, the selfed of selfed group was compared with each of the other classes of plants with the following results:

Class	$\frac{\text{Diff.}}{\text{P.E.}}$
Selfed of crossed	2.77
Crossed of selfed	10.14
Crossed of selfed and crossed	16.32
Crossed of crossed	10.85

These results then indicate that there is no significant difference between the standard deviation in diameter of pollen grains in the selfed of selfed, and selfed of crossed classes. On the other hand, there is a very significant difference between that of the above 2 classes and that of the crossed of selfed, crossed of selfed and crossed, and crossed of crossed groups. As has been shown above, a significant difference in standard deviation indicates a

significant difference in the percentage of abnormal pollen. Hence, in the strains studied, it appears that the percentage of poor pollen in plants which are the immediate products of self-pollinations is significantly greater than that in those plants which are the immediate products of cross-pollinations. It is interesting that, although poor pollen is generally considered the result of hybridization, here, among hybrids, self-pollination greatly increases the percentage of abnormal pollen over that found in plants resulting from cross-pollinations.

Summary

1. In the *Nicotiana alata* × *N. Forgetiana* hybrids, pseudo-fertility is exhibited in both self- and cross-sterile combinations, and is of the same order in both cases.

2. It has been definitely demonstrated that in *Nicotiana* pseudo-fertility is not of the same order as true fertility, but that it does stand in direct relation to true sterility.

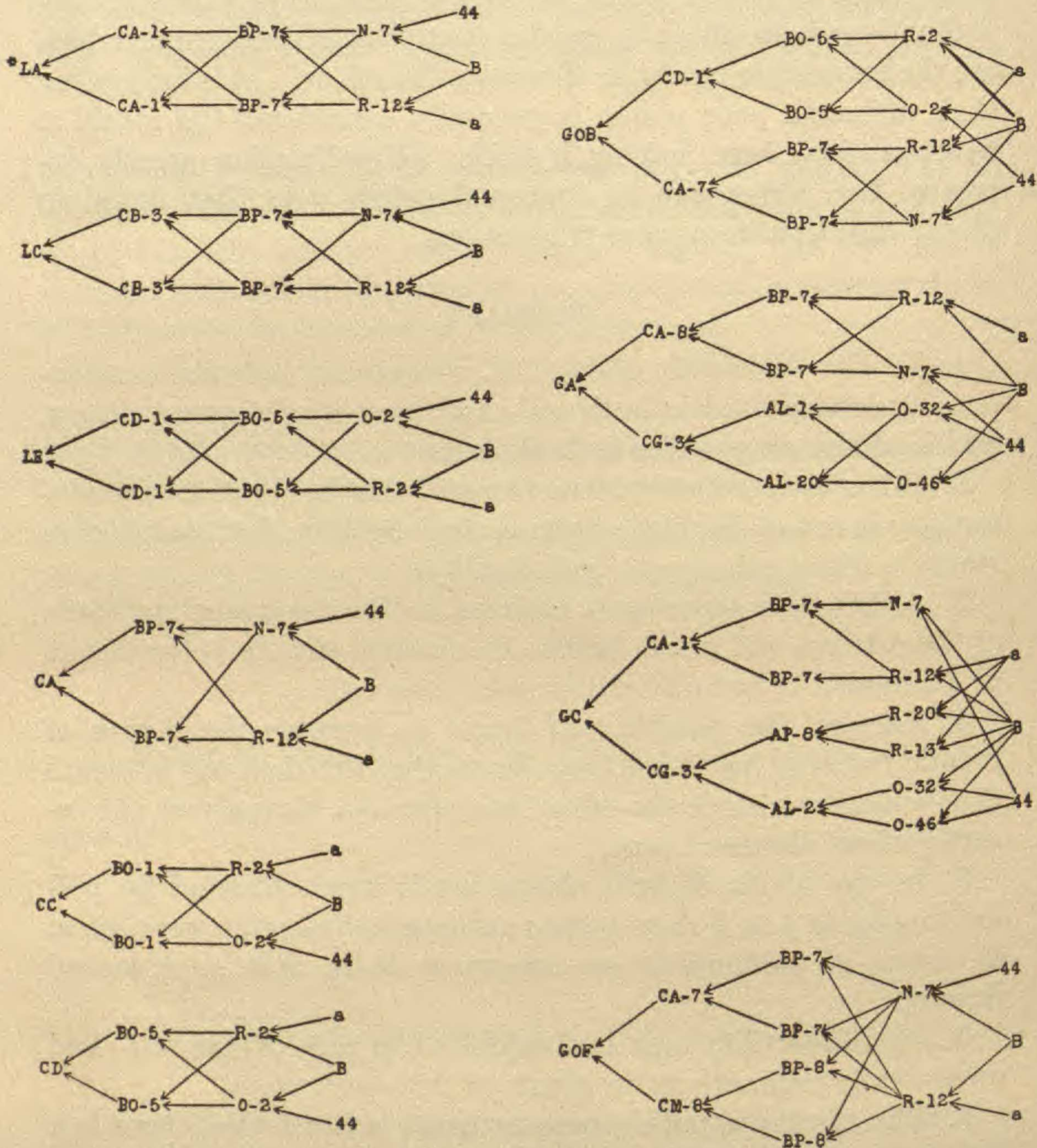
3. Although in some cases, perhaps, pollinations were not made at the extreme end of the season, no seasonal change in the degree of expression of pseudo-fertility was observed.

4. Bagging the panicle was found to increase the degree of pseudo-fertility, but it has been shown that this does not influence the conclusions, since the effect was constant throughout the investigations discussed here.

5. In one of the hybrid plants, seeds were obtained by pollinating buds 4 to 6 days before anthesis, when none were set in 17 series of pollinations on unopened buds, first, and second flowers.

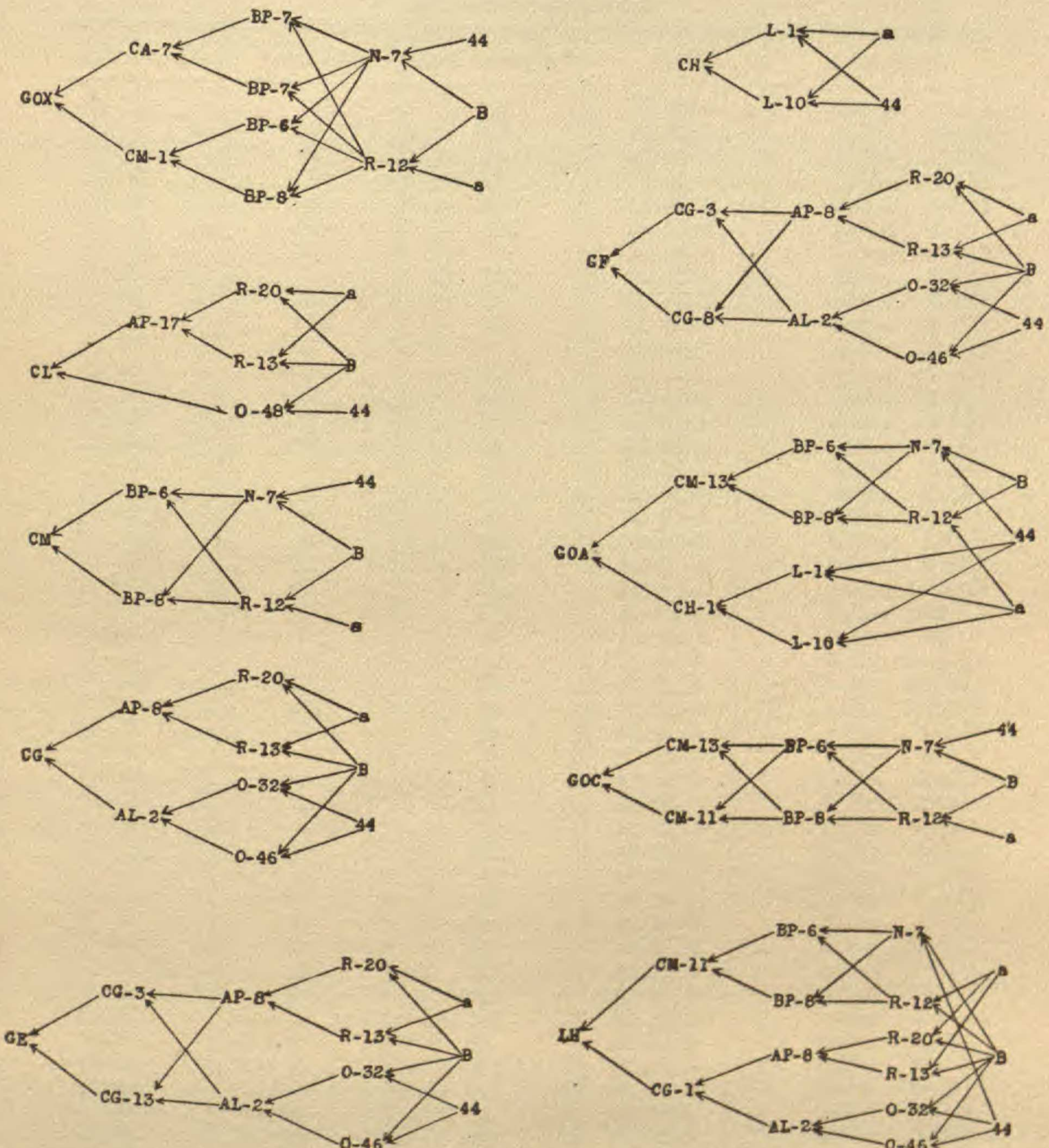
6. Pseudo-fertility was not exhibited in any of the self- and cross-sterile pollinations on plants of *Nicotiana alata*.

7. The results of pollen measurements indicate that there is a larger percentage of abnormal pollen in families arising from self-pollinations than in cross-bred strains.



Pedigree Charts

* The family LA is the result of a self-pollination on plant 1 of family CA, which, in turn, came from a self-pollination on plant 7 of family BP. The family BP, in turn, is the product of a cross between plant 7 of family N, and plant 12 of family R. These families have come from plants 44 and B, and B and a, respectively.



PEDIGREE CHARTS

TABLE VI

COMPLETE DATA

NICOTIANA ALATA × NICOTIANA FORGETIANA

Self-sterile pollinations

Pollination	Date	Unopened bud	1st flower	2nd flower
LA-1 selfed	4-28-24	0	0	0
LA-3 selfed	4-28-24	0	0	0
LC-1 selfed	4-14-24	37	0	0
LC-2 selfed	4-14-24	42	0	0
LC-9 selfed	4-28-24	40	0	0
CA-1 selfed	3-17-23	518	89	0
CA-3 selfed	3-30-23	?	30	0
CB-3 selfed	3-27-23	217	?	56
CB-7 selfed	3-31-23	?	282	26
CB-10 selfed	3-31-23	413	311	27
CB-13 selfed	3-31-23	?	28	81
CB-14 selfed	3-27-23	473+	136+	48+
CB-14 selfed	4-14-23	550	29	54
CC-1 selfed	4-26-23	0	0	0
CC-1 selfed	4-26-23	0	0	0
CC-5 selfed	4-26-23	0	0	0
CC-5 selfed	4-26-23	0	0	0
CC-9 selfed	4- 3-23	0	0	0
CD-1 selfed	4- 5-23	661	0	0
CD-4 selfed	4- 5-23	588	27	?
GOB-1 selfed	4-16-24	387	79	120
GOB-2 selfed	4-18-24	326	217	0
GOB-4 selfed	4-21-24	0	0	0
GOB-4 selfed	4-30-24	314	0	0
GOB-6 selfed	4-28-24	491	262	211
GOB-7 selfed	4-21-24	0	168	0
GA-1 selfed	4-21-24	0	0	0
GA-2 selfed	4-18-24	0	0	0
GA-2 selfed	4-28-24	0	0	0
GA-2 selfed	4-30-24	27	0	0
GA-3 selfed	4-28-24	0	0	0
GA-4 selfed	4-28-24	284	116	0
GA-5 selfed	4-30-24	0	0	0
GA-6 selfed	4-30-24	0	0	0
GC-1 selfed	3-29-24	48	0	0
GC-1 selfed	4- 8-24	14	0	0
GC-2 selfed	4-26-24	123	0	0
GC-3 selfed	4-14-24	124	0	0
GC-3 selfed	4-26-24	387	0	0
GC-4 selfed	4-14-24	62	0	0
GC-4 selfed	4-16-24	140	0	0
GC-5 selfed	4-14-24	29	0	0
GC-6 selfed	4-18-24	0	0	0
GC-6 selfed	4-26-24	149	0	0
GC-9 selfed	4-26-24	100	0	0
GC-12 selfed	4-18-24	0	0	0
GOF-2 selfed	4-21-24	75	0	0
GOF-3 selfed	4-18-24	0	0	0
GOF-3 selfed	4-30-24	0	0	0
GOF-4 selfed	4-28-24	0	103	0
GOF-4 selfed	4-30-24	4	4	13
GOF-5 selfed	4-21-24	0	0	0

Pollination	Date	Unopened bud	1st flower	2nd flower
GOX–1 selfed	4– 8–24	0	0	0
GOX–1 selfed	4–11–24	0	0	0
GOX–3 selfed	4–30–24	0	0	0
GOX–5 selfed	4–30–24	0	0	0
GE–1 selfed	4–21–24	0	0	0
GE–1 selfed	4–28–24	0	0	0
GF–1 selfed	4–16–24	0	0	0
GF–1 selfed	4–28–24	329	0	0
GF–1 selfed	4–30–24	0	0	0
GF–2 selfed	4–16–24	121	0	0
GF–2 selfed	4–28–24	0	0	0
GF–3 selfed	4–30–24	0	0	0
GF–5 selfed	4–30–24	0	0	0
GF–6 selfed	4–21–24	146	0	0
GF–7 selfed	4–21–24	31	371	0
GF–9 selfed	4–21–24	0	208	0
GF–9 selfed	4–28–24	0	0	0
GF–8 selfed	4–30–24	117	0	0
GOC–1 selfed	5– 1–24	0	0	0
GOC–3 selfed	4–30–24	76	0	0
CH–2 selfed	3–27–23	0	0	0
CM–12 selfed	4–14–23	0	0	0
GOA–1 selfed	4–11–24	0	0	0
GOA–1 selfed	4–14–24	0	0	0
GOA–1 selfed	4–30–24	0	0	0
GOA–2 selfed	4–16–24	419	154	0
GOA–2 selfed	4–30–24	7	0	0
GOA–3 selfed	4–18–24	0	0	0
GOA–3 selfed	4–30–24	0	0	0
GOA–4 selfed	4–21–24	0	0	0
GOA–4 selfed	4–30–24	0	0	0
LH–1 selfed	4–21–24	67	0	0
LH–1 selfed	4–28–24	45	79	0
CG–1 selfed	4– 6–23	0	0	0
CG–5 selfed	3–27–23	24	0	0
CG–9 selfed	4– 7–23	54	0	0
CG–10 selfed	3–27–23	0	0	0
CG–18 selfed	4– 5–23	?	?	0
CG–20 selfed	4– 7–23	?	0	0

Cross-sterile pollinations

Pollination	Date	Unopened bud	1st flower	2nd flower
LC–2 × LC–4	4–29–24	72	0	0
CA–3 × CA–1	3–30–23	?	46	0
CA–3 × CA–4	3–30–23	?	69	0
CA–13 × CA–11	3–25–23	378	307	155
CA–4 × CB–13	4–19–23	338	0	55
CA–4 × CB–13	4–19–23	158	0	0
CA–6 × CB–4	4–10–23	?	195	314
CA–6 × CB–9	4–10–23	385	0	0
CA–11 × CB–15	3–27–23	379	231	73
CA–12 × CB–9	4–10–23	250	0	0
CB–7 × CB–5	3–31–23	?	0	0
CB–7 × CB–14	3–31–23	?	98	?
CB–12 × CB–6	3–27–23	?	?	0
CB–13 × CB–10	4– 5–23	0	0	0

Pollination	Date	Unopened bud	1st flower	2nd flower
CB-13 × CB-14	3-31-23	21	0	0
CB-14 × CB-15	4- 5-23	538	276	0
CB-14 × CA-4	4-14-23	418	31	?
CC-1 × CC-5	4-26-23	355	390	338
CC-1 × CC-5	4-26-23	461	381	261
CC-5 × CC-1	4-26-23	0	0	0
CC-5 × CC-1	4-26-23	0	?	?
CC-6 × CC-9	4-26-23	0	164	0
CC-7 × CC-9	4- 3-23	?	0	0
CC-8 × CC-7	4- 7-23	261	?	0
CC-9 × CC-7	4- 7-23	0	0	205
CD-1 × CD-2	4- 5-23	536	0	?
GA-3 × GA-4	4-18-24	0	0	0
GA-3 × GA-6	4-28-24	0	0	0
GA-5 × GA-2	4-28-24	194	0	0
GA-6 × GA-4	4-28-24	257	0	0
GA-15 × GA-16	4-30-24	0	0	0
GC-3 × GC-4	4-18-24	183	?	0
GC-5 × GC-7	4-21-24	248	0	0
GC-9 × GC-4	4-26-24	0	0	0
GF-7 × GF-9	4-28-24	530	136	47
GF-9 × GF-18	4-30-24	8	0	0
GOC-1 × GOC-3	4-30-24	0	0	0
GOC-3 × GOC-1	4-30-24	63	0	0
CM-8 × CM-3	4- 7-23	461	0	0
CM-8 × CM-6	4- 7-23	141	?	0
CM-11 × CM-3	4-14-23	250	0	0
CM-12 × CM-5	4-14-23	0	0	0
CG-2 × CG-10	4- 3-23	424	0	0
CG-5 × CG-10	3-27-23	?	?	?
CG-6 × CG-5	4- 7-23	?	0	0
CG-6 × CG-8	4- 7-23	0	0	23
CG-8 × CG-10	4- 5-23	470	41	32
CG-9 × CG-1	4- 3-23	0	0	0
CG-10 × CG-2	3-27-23	0	0	0
CG-10 × CG-2	4- 3-23	0	0	0
CG-18 × CG-8	4- 7-23	?	0	0
CG-19 × CG-4	4 -5-23	0	0	0
CL-3 × CL-5	4- 7-23	36	0	0
CL-4 × CL-10	4- 5-23	72	0	0
GOA-1 × GOA-2	4-23-24	0	0	0
GOA-2 × GOA-4	4-26-24	322	0	10
GOA-3 × GOA-1	4-26-24	0	0	0
GA-5 × GC-6	5- 1-24	0	0	0
GE-1 × GOF-1	4-28-24	4	0	0
GF-7 × GC-9	4-30-24	0	0	0
GOA-1 × GE-1	4-23-24	11	0	0
GOA-4 × GOB-1	4-26-24	289	0	?
GOA-1 × GOX-1	4-18-24	120	0	0
GOB-1 × GOA-4	4-26-24	161	0	0
GOC-1 × GOA-2	4-26-24	0	0	0
GOC-1 × GOX-1	4-26-24	0	0	0
GOF-5 × GOX-3	4-28-24	0	0	0
GOX-1 × GOA-4	4-26-24	0	0	0
LA-1 × LC-6	4-28-24	243	0	0
LH-1 × GI	4-28-24	76	37	409
LH-1 × LA-1	4-28-24	0	0	0
LH-1 × LC-5	4-28-24	75	0	0
CB-4 × CG-8	4-19-23	230	87	41

Pollination	Date	Unopened bud	1st flower	2nd flower
CB–4 × CG–8	4–19–23	292	105	0
CC–1 × CL–4	4–26–23	?	413	26
CC–5 × CL–4	4–26–23	0	0	0
CC–5 × CL–4	4–26–23	124	?	0
CD–4 × CG–20	4– 5–23	519	0	0
CG–20 × CL–4	4–19–23	334	0	0
CG–20 × CL–4	4–19–23	0	0	0
CH–1 × CM–6	3–31–23	?	0	0
CM–12 × CH–2	4–14–23	0	0	0
CG–5 × CB–10	3–27–23	0	0	0

Cross-fertile pollinations

Pollination	Date	Unopened bud	1st flower	2nd flower
LC–4 × LC–3	4–28–24	48	?	216
LC–5 × LC–1	4–28–24	422	153	187
GOB–7 × GOB–3	4–28–24	540	?	390
GA–3 × GA–15	4–28–24	371	274	?
GA–6 × GA–8	4–28–24	342	378	285
GA–6 × GA–15	4–28–24	357	343	225
GC–5 × GC–9	4–16–24	385	437	430
GC–9 × GC–3	4–26–24	522	222	627
GOF–3 × GOF–1	4–21–24	393	320	279
GOF–5 × GOF–13	4–30–24	181	245	430
GOX–1 × GOX–4	4–26–24	359	411	502
GOX–3 × GOX–4	4–26–24	288	475	511
GF–1 × GF–10	4–28–24	291	396	379
GF–7 × GF–10	4–30–24	528	646	591
GF–9 × GF–10	4–28–24	494	390	369
CM–1 × CM–3	4– 7–23	689	669	0
CM–1 × CM–12	4– 7–23	0	666	0
CM–1 × CM–14	4– 7–23	254	455	321
CM–11 × CM–14	4– 7–23	500	?	409
CG–3 × CG–8	4– 7–23	301	248	243
CG–5 × CG–7	4– 3–23	362	334	410
CG–6 × CG–1	4– 3–23	838	969	825
CG–18 × CG–9	4– 5–23	?	530	488
CL–2 × CL–1	4– 7–23	481	515	455
CL–5 × CL–10	4– 7–23	467	458	418
CL–8 × CL–1	4– 7–23	311	780	713
CL–8 × CL–6	4– 7–23	787	834	724
CL–10 × CL–2	4– 7–23	321	396	446
GC–3 × GOA–1	4–14–24	433	685	703
GOB–1 × GA–1	4–30–24	293	422	295
GOE–1 × GOF–1	4–18–24	381	171	101
GOF–4 × GOA–4	4–28–24	234	80	341
CA–3 × CH–1	3–30–23	?	673	327
CB–14 × CH–2	4–14–23	444	396	484
CB–14 × CM–12	4–14–23	246	256	247
CB–15 × CM–12	3–27–23	315	211	0
CD–1 × CA–1	4– 5–23	1004	873	908
CD–4 × CL–5	4– 5–23	681	398	599
CD–4 × CM–6	4–14–23	257	570	496
CF–3 × CH–1	3–27–23	619	343	515
CG–20 × CA–1	4– 7–23	511	577	446
CH–1 × CG–18	3–31–23	757	586	649
CH–2 × CA–4	4–14–23	388	395	?
CH–2 × CB–14	4–14–23	356	377	342
CH–2 × CL–6	4–14–23	440	439	429
CM–11 × CG–1	4– 3–23	?	553	?
CM–12 × CA–4	4–14–23	424	646	24
CM–12 × CB–14	4–14–23	750	612	431
CM–12 × CL–6	4–14–23	500	717	627

NICOTIANA ALATA				
Self-sterile pollinations				
Pollination	Date	Unopened bud	1st flower	2nd flower
CJ-3 selfed	4- 3-23	0	0	0
HA-3 selfed	4-18-24	0	0	0
HA-3 selfed	5- 1-24	0	0	0
HA-4 selfed	4-29-24	0	0	0
HA-5 selfed	4-21-24	0	0	0
HB-1 selfed	4-19-24	0	0	0
HC-2 selfed	4-21-24	0	0	0
HC-2 selfed	4-29-24	0	0	0
HC-3 selfed	4-29-24	0	0	0
HI-1 selfed	4- 8-24	0	0	0
HI-2 selfed	4-14-24	0	0	0
HI-3 selfed	4-14-24	0	0	0
HI-3 selfed	5- 1-24	0	0	0
HI-4 selfed	4-16-24	0	0	0
HI-5 selfed	4-18-24	0	0	0
HI-8 selfed	4-21-24	0	0	0
Cross-sterile pollinations				
CJ-1 × CJ-3	4- 3-23	0	0	0
CJ-2 × CJ-1	4- 3-23	0	0	0
HA-5 × HA-6	4-29-23	0	0	0
HC-2 × HC-1	4-14-24	0	0	0
HC-2 × HC-1	4-29-24	0	0	0
HI-2 × HI-9	4-29-24	0	0	0
Cross-fertile pollinations				
HA-1 × HA-5	4-29-24	679	600	605
HA-5 × HA-3	4-29-24	600	663	500
HA-6 × HA-3	4-29-24	637	737	831
HC-1 × HC-5	4-29-24	155	446	258
HC-2 × HC-4	4-29-24	1000	13	936
HA-I × HI-9	4-18-24	787	654	773
HA-5 × HI-2	4-24-24	900	1569	1079
HB-1 × HC-1	4-21-24	450	500	1000

TABLE VII

POLLEN MEASUREMENTS (IN TERMS OF DIVISIONS ON MICROMETER SCALE: 1 DIVISION = 8 MICRA)

	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0
CA-7					4		17		3		1		
CA-13					4		16		4		1		
CB-1			2		4		13		5		1		
CB-4			3		3		14		3		2		
CB-5					7		8		10				
CB-7	1		6		6		5		6		1		
CC-1					3		11		7		4		
CC-2	1		4		3		10		4		2		1
CC-3			1		1		10		8		3		2
CC-4					2		14		9				
CC-5			1		1		16		5		2		

	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0
CC–8			2		3		7		7		6		
CC–10			1		3		8		10		3		
CC–11			1		3		17		3		1		
CD–1	1		5		2		8		9				
CD–2	2		4		3		13		3				
CD–3	1		4		7		13						
CD–4	3		6		4		10		2				
CD–8	1		1		4		15		4				
CL–4					3		8		13		1		
CL–7					9		16						
CL–8			3		6		16						
CL–11					3		22						
CM–1					2		20		3				
CM–6					2		21		2				
CM–10					1		19		5				
CM–13					2		19		4				
CG–20					9		16						
CH–1					1		22		1			1	
GA–1			1	6	14	20	8	1					
GA–2			1	3	15	25	6						
GA–3				4	8	22	15	1					
GA–4			1	1	13	23	10	2					
GA–5			1	4	2	27	14	2					
GA–6			2	3	7	22	14	2					
GA–8				1	4	20	24	1					
GA–15				2	7	23	16	2					
GA–16			2	3	10	19	13	3					
GC–2					10	16	24						
GC–3				1	10	17	19	3					
GC–4			3	7	15	11	14						
GC–6				3	6	16	25						
GC–7	2		5	7	14	17	5						
GC–9	3		5	1	10	10	19	2					
GC–13			2	1	10	10	26	1					
GE–1	3		2	2	6	17	20						
GF–1				1	10	23	16						
GF–2	2		3	2	3	23	16	1					
GF–3				2	5	13	26	4					
GF–4	2		3	3	4	22	7	4	5				
GF–5			3	6	8	25	7	1					
GF–6	1		1	2	4	15	25	2					
GF–7			1	2	3	20	22	2					
GF–8				1	6	18	25						
GF–9					6	28	16						
GF–10				2	4	23	20	1					
GF–11			1	2	4	26	17						
GF–18		1	1	1	3	24	20						
GOA–1		1	1	2	8	25	12	1					
GOA–2			6	8	11	12	11	2					

	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0
GOA-3				2	10	17	20	1					
GOA-4		1	3	10	13	15	7	1					
GOA-5			2	2	2	10	21	10	1	1	1		
GOA-6	8	20	8		1	5	7		1				
GOA-7				1	3	8	18	19	1				
GOB-1				1	2	26	20	1					
GOB-2			1	1	10	25	12	1					
GOB-3		2	7	3	6	10	20	2					
GOB-4		4	7	1	4	11	22	1					
GOB-6	1	2	3	4	6	19	12	2	1				
GOB-7			3	4	4	17	19	3					
GOB-8		2	3	4	6	17	15	2	1				
GOC-1		3	2	2	6	17	20						
GOC-3			1	1	6	21	17	3	1				
GOC-10				5	11	24	9	1					
GOC-11			1	2	4	7	18	17	1				
GOF-1					13	27	9	1					
GOF-3	4	2	5	3	3	22	11						
GOF-4			1	4	17	24	2	1	1				
GOF-5	2	5	1	1	13	23	5						
GOF-6			2	4	17	11	9	6	1				
GOF-7	4	3	1	2	22	16	2						
GOF-11			1	2	4	26	17						
GOF-13	4	1	4	3	19	19							
GOX-1				3	10	18	19						
GOX-2			2	2	10	21	14	1					
GOX-3			1	2	2	20	15	7	3				
GOX-4			1	2	5	22	17	3					
GOX-5				6	10	22	12						
GOX-6				1	10	20	19						
LA-1	2	1	2	1	4	13	14	6	5	2			
LA-3	2	1	2	2	1	15	19	5	2	1			
LA-4	3	4	5	5	5	7	17	3	1				
LC-5	2	2	2	5	4	10	13	3	4	3	1	1	
LC-6	2	3	3	5	7	13	11	2	2	1	1		
LC-8		3	5	6	7	9	10	4	4	1	1		
LC-9		3	3	4	4	11	15	4	3	1	1	1	
LE-3	1	1	2	1	5	18	17	3	2				
LE-5				3	11	25	11						
LH-1	3	5	7	5	6	13	10	1					
LH-3	1	3	5	5	8	18	10						

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